Effect of arbuscular mycorrhiza on the growth of *Camptotheca acuminata* seedlings

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Abstract: *Camptotheca acuminata* seeds were sown in sterilized sands in the greenhouse in February of 2005. After 90-day growth, seedlings were inoculated with three species of arbuscular mycorrhizal fungi (AMF), *Acaulospora mellea*, *Glomus diaphanum* and *Sclerocystis sinuosa.*. The height, biomass, and absorptions of nitrogen and phosphorus of *C. acuminata* seedlings inoculated with AMF were investigated. The results showed that the formation of AM promoted the height growth and biomass accumulation of seedlings significantly and improved the absorption of phosphorus in seedlings. The height and biomass of mycorrhizal seedlings were 1.2 and 1.6 times higher than those of the non-mycorrhizal seedlings. The absorption of nitrogen was less influenced by the formation of AM. The nitrogen content in mycorrhizal seedling was equal to that of non-mycorrhizal seedlings. Compared with non-mycorrhizal seedlings, the nitrogen content of mycorrhizal seedlings inoculated with *A. mellea* changed considerably in the root, stem and leaves. The difference in nitrogen content was not significant between mycorrhizal seedlings inoculated with *G. diaphanum* and *S. sinuosa*. The AM formation stimulated the absorption of phosphorus, especially in roots, and also changed the allocation of nitrogen and phosphorus in different organs of seedlings. Compared with non-mycorrhizal seedlings, the ratio of nitrogen and phosphorus in mycorrhizal roots increased, but reduced in stem and leaves.

Keywords: Arbuscular mycorrhiza (AM); *Camptotheca acuminata* seedlings; Biomass; Nitrogen and phosphorus contents

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Introduction

Arbuscular mycorrhiza (AM), a mutualistic symbiont of plant and fungi, widely exists in the nature, and about 80%-90% angiosperm can form AM (Koide 1992). AM may enhance water absorbing ability of plant from soil (Bi 2001), improve the absorption of plant root system for nutrients and mineral elements of phosphorus, copper, zinc and cadmium (Hamel 1996, Wang 2003), and promote the plant growth (Mahendra 2001). Zandavalli (2004) studied *Araucaria angustifolia* inoculated with *Glomus clarum*, found that the biomass and nitrogen content of *A. angustifolia* forming AM increased significantly.

Camptotheca acuminata, a special tree species in China, has drawn a great attention because its secondary metabolite (camptothecin (CPT)) has remarkable anti-tumour activity (Yan 2003). In recent years, some studies have been conducted on this species, but these studies mostly focused on the effects of environmental factors on growth of *C. acuminata* (Zhang 2000). However, little attention has been paid to the effects of biological factors on the growth of *C. acuminata* seedling.

In 2004, we performed a preliminary experiment and investigated the inoculating efficiencies and secondary metabolic responses of six AM fungi species for *C. acuminata* seedlings, including *Acaulospora mellea*, *A. laevis*, *Glomus manihot*, *G. versiforme*, *G. etunicatum* and *G. diaphanum* belonging to two genera. On the basis of the results, two fungi with the most positive effects and one fungus from a new genera were used to further study their influences on the growth of *C. acuminata* seedlings and the absorption for nitrogen and phosphorus in 2005.

Materials and methods

Arbuscular mycorrhizal fungi (AMF)

The fungi of *Glomus diaphanum*, *Acaulospora mellea* and *Sclerocystis sinuosa* were obtained from Beijing Academy of Agriculture and Forestry Sciences, Institute of Soil Science of Chinese Academy of Sciences, and Soil and Fertilizer Institute of Chinese Academy of Agricultural Sciences, respectively.

Seedling cultivation and inoculation treatments

The seeds of *C. acuminata* sterilized with 0.5% $KMnO_4$ were washed with sterilized water firstly, then sown in sands that had been sterilized in 121ºC for 2 h. As the lateral root germination, seedlings were moved into pots (with a diameter of 20 cm) filled with the mixture of soil and sand (V:V=3:1) screened with sifter (a diameter of 2 mm) and sterilized in 121 ºC for 2 h, in which, the organic matter content, total nitrogen content, available nitrogen content, and available kalium content were 1.93%, 1.16 $g\cdot kg^{-1}$, 13.41 $mg\cdot kg^{-1}$, 0.24 $g\cdot kg^{-1}$, respectively. And pH of the mixture of soil and sand was about 6.42.

About 90 days later, those seedlings with similar height and crown were selected and divided into four groups (10 pots in each group). Three of them were inoculated with 30 g AM inocula of *Glomus diaphanum*, *Acaulospora mellea* and *Sclerocystis sinuosa* (Ss), and the other without AM inoculation was the control (CK). All these seedlings were harvested on three months later.

Mycorrhizal colonization

The mycorrhizal colonization frequency (F%), root mycorrhizal colonization intensity (M%), and arbuscular abundance were calculated by the method of Trypan Blue Staining (Trouvelot 1986). The mycorrhizal colonization frequency is the proportion of the roots containing fungal structure to the whole roots system. Root mycorrhizal colonization intensity (M%) shows the intensity

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of mycorrhizal fungi infection, and the arbuscule abundance is the richness of arbuscule structure in the whole root system (A%).

Determinations of the total nitrogen and phosphorus

Total nitrogen content was determined by Auto-Kjeldahl method (Cui 1998), and total phosphorus content was determined by the colorimetric method of sulfuric-perchloric acid (Cui 1998).

All data were analyzed by the software of Statistical Product and Service Solutions (SPSS).

Results and analysis

Mycorrhizal formation of *C. acuminata* seedlings

All seedlings of *C. acuminata* inoculated with three AM fungi were infected and formed arbuscular mycorrhiza in various degrees (Table 1). The mycorrhizal colonization frequencies (F%) of the three AM fungi were all more than 70%. Mycorrhizal colonization intensity of roots (M%) and arbuscule abundance (A%) were also relatively high. These results indicated that symbiosis systems were well formed between *C. acuminata* seedlings and selected mycorrhizal fungi.

Table 1. Colonization status of *C. acuminata* inoculated with three kinds of arbuscular mycorrhizal fungi

Treatments	Mycorrhizal colonization frequency (%)	Mycorrhizal colonization intensity (%)	Arbuscule abundance (%)
Control	0	0	0
Glomus diaphanum	78.44 ± 2.96	30.76 ± 4.24	30.20 ± 5.60
Acaulospora mellea	93.26 ± 5.28	48.39 ± 6.13	43.97 ± 4.02
Sclerocystis sinuosa	95.63 ± 4.38	50.29 ± 1.78	44.20 ± 5.25

Effect of AM on *C. acuminata* seedlings growth

After 100 days inoculation, the formation of AM had an obvious effect on the growth of *C. acuminate* seedlings. The height growth of all the mycorrhizal seedlings was faster than that of non-mycorrhizal seedlings (control). The height of mycorrhizal seedlings inoculated with *A. mellea* was 1.2 times of the control (Table 2).

The biomass of most of mycorrhizal seedlings was significantly greater than that of the control (Table 2). Of three treatments, the biomass of the seedlings inoculated with *A. mellea* was the greatest, which is 160% higher than that of the control. Moreover, the difference among mycorrhizal seedlings was significant (Table 2).

Table 2. Effect of arbuscular mycorrhiza on the growth of *C. acuminata* seedlings

Treatments	Height (cm)	Biomass ($g \cdot plant^{-1}$)	Root/shoot ratio
Control	18.78^{a}	3.12^{a}	0.62^{a}
Glomus diaphanum	20.61^{b}	4.06^{b}	0.62^{a}
Acaulospora mellea	23.25^{c}	4.92^{c}	0.73^{b}
Sclerocystis sinuosa	22.58^{c}	4.40^{bc}	0.67^{a}

Note: Data with different letters have significantly difference ($p<0.05$) in the same column.

The ratio of root to shoot has been taken as a standard in evaluating the growth of seedlings and the efficiency of absorbing nitrogen and phosphorus in plants. In this study, it was observed that the general biomass allocation between root and shoot in seedlings was less affected by the formation of AM, except for the *A. mellea* (Table 2).

Effects of AM on the absorption of nitrogen and phosphorus in *C. acuminata* seedlings

AM had no obviously effect on absorption of nitrogen in *C. acuminata* seedlings. The nitrogen contents of the seedlings inoculated with *G. diaphanum* and *S. sinuosa* in root, stem and leaves were slightly higher than that of the control (Fig. 1), and the differences of nitrogen contents among them were not significant. The nitrogen content in the seedlings inoculated with *A. mellea* had obviously changes, which were 1.3 and 1.1 folds higher than that of the control in root and stem, respectively. On the contrary, nitrogen content of leaves in the seedlings inoculated with *A. mellea* was significantly lower than that of the control. The nitrogen contents in whole plant had not significant difference between the seedlings inoculated with different fungi and the control seedlings.

The effect of AM formation on phosphorus absorption was different with nitrogen absorption. As a whole, AM promoted the phosphorus absorption (Fig. 1). No significant effect of AM on the phosphorus contents in stem and leaves was observed, but in root. The phosphorus contents in root of the seedlings inoculated with *G. diaphanum, A. mellea,* and *S. sinuosa* were 2.2, 3.0 and 3.2 times higher than that of the control, respectively, and the difference was significant among all mycorrhizal seedlings. For the whole plant, phosphorus contents in the seedlings inoculated with *G. diaphanum, A. mellea,* and *S. sinuosa* were 1.5, 1.8 and 1.9 times higher than that of the control seedlings, respectively.

Furthermore, the allocation of nitrogen and phosphorus in distinct organs of seedlings were also affected by formation of arbuscular mycorrhizal. The ratio of nitrogen allocation in roots of the seedling inoculated with *G. diaphanum* and *S. sinuosa* increased slightly comparing with that of the control, but it is increased significantly in the seedlings inoculated with *A. mellea* (Fig. 2). The ratios of nitrogen allocation in stems and leaves of all mycorrhizal seedlings were lower than those of the control.

The phosphorus allocation in various organs of seedlings was also affected by AM, which is not similar to thec hanges of nitrogen allocation. The ratios of phosphorus allocation in all mycorrhizal roots were significantly higher than those in the control, while the allocations in stems and leaves were lower than those in non-mycorrhizal seedlings (Fig. 2).

Discussion

The effects of inoculation with arbuscular mycorrhizal fungi on the growth and development of young *Macadamia* plants at the nursery had been investigated by Liu (2005). Their results showed that the AMF could promote the seedling growth, improve the absorbency of root, shoot and root growth, increase the root/shoot ratio and the phosphorus content in the plant tissues. Plenchette and Duponnois (2005) reported that the formation of AM stimulates the absorption of phosphorus, but not nitrogen, in host plant. The similar results were observed in this study that the formation of AM significantly promoted the *C. acuminata* seedling growth and biomass accumulation and improved the phosphorus absorption in seedlings, but the absorption of nitrogen was little affected by the AM infection. Compared with non-mycorrhizal seedlings, the ratio of nitrogen and phosphorus allocation of mycorrhizal seedlings increased in roots, but reduced in stem and leaves. This phenomenon has been also reported on sweet potato (Gai 2004).

Moreover, the effect of AM fungi on absorption of nitrogen and

phosphorus in *C. acuminata* seedlings was different with various genera of AM fungi.

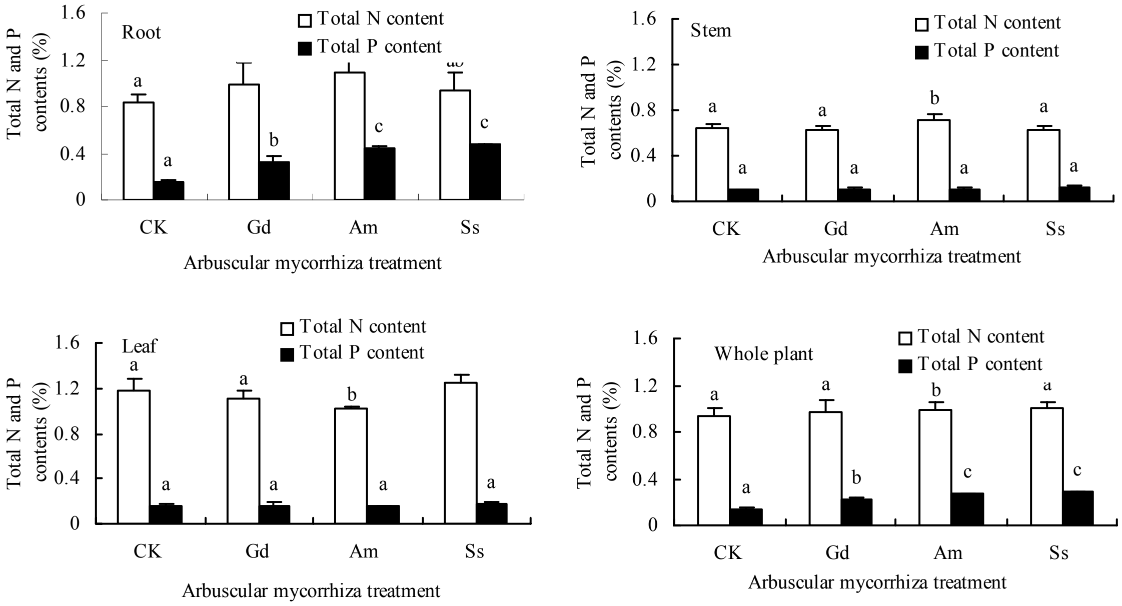

Fig.1 N and P contents of *C. acuminata* seedlings with different arbuscular mycorrhiza
In each panel, the bars with different letters are significantly different (p<0.05)
CK---control; Gd--- *Glomus diaphanum*, Am---*Acaulospora mellea,* Ss--- *Sclerocystis sinuosa*

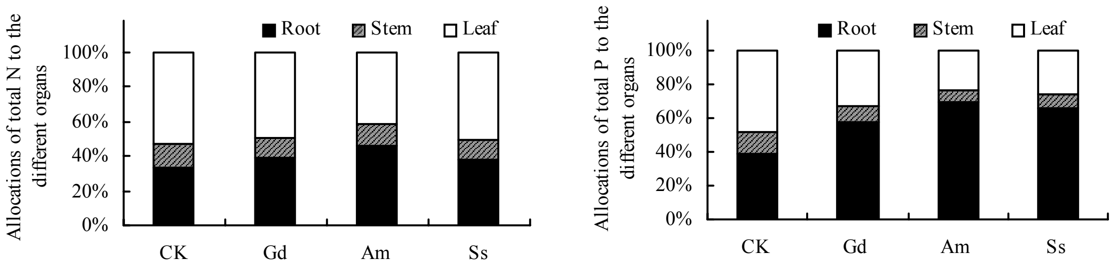

Fig.2 Allocations of N and P to the different organs of *C. acuminata* seedlings with different arbuscular mycorrhiza
CK---control, Gd--- *Glomus diaphanum*, Am---*Acaulospora mellea,,* Ss--- *Sclerocystis sinuosa*

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